Kozo Nishio*: Investigation of vascular plants with the Mäule test**

西尾康三*: モイレ反応による維管束植物の研究

This work is an observation of groups of plants in the level of tissues in application of the Mäule test for the purpose of throwing some light on the study of evolution in the plant kingdom.

A color reaction based upon a pretreatment with chlorine followed by a weak base is known as "the Mäule test for lignin", taking the name of the person who found this reaction (Mäule, 1901). Mäule obtained a deep rose-red color with woods of several species including *Selaginella caesia.* He noted that the woods of gymnosperms reacted less strongly. He did not realized that his test may be used in general to distinguish between angiospermous and gymnospermous woods.

After Mäule, the fact that red color (positive) is shown by angiosperms and yellow brown or yellow color (negative) is shown by gymnosperms was stated by Lamarlière (1903), Ungar (1914), Schorger (1917) (Reference Gibbs, 1957), Crocker (1921) and Sharma (1922). (Sharma tested 29 gymnosperms and found them constantly negative, while 41 angiosperms gave positive reactions).

After these researches, some exceptional cases were found. Siersch (1926) found positive colors in 4 out of 6 species of *Selaginella*, one species out of 6 cycads (*Zamia integrifolia*) and one species of the Gnetales (a species of *Ephedra*). Schindler (1931) examined all 3 genera of the Gnetales and found *Gnetum gnemon*, *Welwitschia mirabilis* and 2 species of *Ephedra* to be positive. A further paper by Crocker (1933) reported that one species of *Podocarpus* (*P. amarus*) gave a positive reaction.

Campbell et al. (1938) stated that the red color given by hardwood is caused by the occurrence of a syringil group in the lignin molecule of these woods. Creighton et al. (1944) tested 50 species of pteridophytes, gymnosperms, mono- and dicotyledons with the Mäule test and compared the results with those obtained on oxidation with nitrobenzene, whereby either vanillin alone, or a mixture of vanillin and syringaldehyde, was obtained. With each gymnosperm that gave vanillin only, the Mäule test was negative; with angiosperms that gave a mixture of vanillin and syringaldehyde, the Mäule test was positive.

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Gibbs (1957) made a comprehensive review of works supplemented with the result of his own work. He manifested in the tables in his review the occurrence of oxidation products—para-hydroxybenzaldehyde, vanillin and syringaldehyde, and the ratio of syringaldehyde to vanillin in the oxidation products in many kinds of plants belonging to different groups. He confirmed the relationship between the chemical structure of lignin and the Mäule reaction stated by Campbell et al. Furthermore, he discussed the relationship of Mäule reaction to the taxonomical position of plants tested. In pteridophytes he found noticeable positive colors only in the genus *Selaginella*. In gymnosperms the noticeable positive colors were shown in some of the Cycadaceae; in some of *Podocarpus* and in all the Gnetales tested. Virtually all dicotyledons gave positive reactions. He suggested that the presence of the syringil radical in large amount would seem to be a character which has come into existence several times. He also pointed out utility of the Mäule reaction to plant taxonomy by showing examples in the genus *Acer*. He is the first person who directed this observation of the Mäule test on the level of tissues, although the observation was limited.

According to the preceding researchers, it has been generally accepted that lignin of the Mäule negative reaction is predominantly produced by pteridophytes and gymnosperms, while lignin of the Mäule positive is predominantly produced by angiosperms. They also reported some cases of exception and many grades of intermediate in syringaldehyde/vanillin ratio between 0 to 5.2.

Recent advance in plant phylogeny has made us believe that the origin of pteridophytes and gymnosperms is polyphyletic. Furthermore, origination and evolution of vessels, which had been recognized for its important value in phylogeny of vascular plants, were stated as polyphyletic by Bailey (1944).

In 1959, the author applied the test to those plants which would supposedly be intermediate between the coniferyl type and the syringil type in lignification (Nishio (1959a)). Tissues of those plants very often showed various grades in color from red to yellow in the same plant. In *Podocarpus macrophylla*, this occurred in the xylem, showing stripes of red and yellow. In *Illicium anisatum*, furthermore, both positive and negative colors with the Mäule reaction were provided in the same cell as well as in the same tissue. In the same year, he subjected lower Pteridophytina to the Mäule test (Nishio (1959b)).

No positive color was given in *Psilotum*, *Lycopodium* and *Equisetum*, which were tested. On the other hand, in all 3 species of *Selaginella* tested, positive colors were shown in the xylem and in the cortex. However, the color of reaction in the xylem cells was brownish in its tone.

The author thus engaged in his present work to make observation of vascular plants with the Mäule test in the level of tissue and to arrange the results systematically, referring to perforation of vessels in those plants, hoping that some useful data on the problem of the plant phylogeny would be offered. Eventually, the author put his emphasis on the observation of those dicotyledonous angiosperms which would presumably be primitive.

Materials and methods

Plants used. Pteridophytina: 19 families, 42 genera, 49 species. Gymnospermophytina: 6 orders, 22 genera, 25 species. Angiospermophytina: 114 families, 287 genera, 372 species. All Angiospermophytina plants, which are used in this work, belong to Dicotyledoneae. These plants include: 101 trees, 13 shrubs or trees, 91 shrubs, 121 herbs, 31 woody liana, and 15 herbaceous lianas.

Preparation of samples. Samples to be observed were taken from stems of plants in most cases. Petioles were used in very limited cases (most cases of Pteridophytina) where stems were not available. In using woody plants as samples, the branches of the second year are preferably taken, because the branches of the first year sometimes cause exceptional cases and the older branches are usually too big to be sampled.

The samples to be observed in the living state were prepared by making hand sections. The result of the Mäule test was observed in the transverse sections on the cells which had been known as lignified ones by the reaction with phloroglucinol and hydrochloric acid on sections as similar as possible. In the case of Angiospermophytina, the longitudinal sections were made and perforations between vessels were inspected by staining with the phloroglucinol and hydrochloric acid reaction. As for the perforation, results were collated with Metcalfe and Chalk's Anatomy of Dicotyledons, and the observations were repeated if serious discrepancies were found.

Reagent, treatment and reaction. Phloroglucinol and hydrochloric acid (Wiesner's reagent).

Dissolve 3g phloroglucinol in 100 cc 12% aqueous solution of hydrochloric acid. This reaction gives a purple-red color with lignin Mäule test. Dip a section into 1% aqueous solution of potassium permanganate for 3 minutes. Wash in water for 3 minutes. Repeat this wash twice. Dip a section into 12% aqueous solution of hydrochloric acid for 3 minutes. Wash in water for 6 minutes. Add 25% aqueous solution of ammonium hydroxide. Colors of purple red, red, orange red and reddish orange noted as positive reaction and those of orange, reddish brown, brown, orange yellow, brownish yellow, and yellow as negative reaction.

Results

In pteridophytes, no positive color had been found before, except in *Selaginella*. The author found for the first time positive colors in true ferns (*Plagiogyria japonica*, *Colysis elliptica*, *Neocheiropteris subhastata*, *Elaphoglossum Yoshinagae*, *Monachosorum Arakii* and *Pteridium aquilinum*). There is a tendency that the sclerenchymatous cells in the cortex are more positive in color than the cells in the xylem. It is notable that all the pteridophytes with vessels observed (3 *Selaginella* and 1 *Pteridium*) showed remarkably positive colors.

Among the gymnosperms, *Ephedra distachia* gave a noticeable positive color in the xylem. In *Podocarpus macrophylla*, positive and negative colors were mixed, and predominantly positive parts and predominantly negative parts occurred, showing stripes of red and yellow. The other species showed negative colors with a few exceptions which are not noteworthy.

At the general view of the angiosperms tested, there are four major types represented in the lignification of the xylem: all xylem cells are negative (A), positive and negative colors were mixed in the xylem cells1) (B), only the vessels are negative among the xylem cells (C), and all xylem cells are positive (D).

The plants of the type A are included in Polycarpicae2), Cistifloridae3),

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1) This mosaic in colors very often occurs within a cell as well as a tissue. A good example of this type is *Illicium anisatum*, to which the author referred in the preceding papers of 1959a.

2) This subclass includes Magnoliales, Anonales, Laurales, Ranales and Piperales.

3) This subclass includes Violales, Cucurbitales, Cactales, Tamaricales, Salicales, Rhoeadales, Sarraceniales and Aristolochiales.

and Rosaefloridae[1]. These groups of plants have generally been accepted as the lower one of the Dicotyledoneae. Schisandraceae, Saururaceae, Chloranthaceae and Papaveraceae are dominated by this type among the plants with scalariform perforations in the vessel, and Aristolochiaceae and some herbaceous species of Leguminosae are those which belong to this type among the plants with simple perforations. Fumariaceous plants (2 species were tested), which possess both kinds of perforation in a plant, also belong to this type. Most plants of this type are herbs with weak stems or herbaceous lianas. However, it is notable that *Sarcandra glabra*, *Chloranthus spicatus* and 3 species of Schisandraceae are plants of this type, although they are woody plants.

The type B of lignification are most often found in Ericaceae, Hydrangeaceae, Caprifoliaceae and other groups which consist of shrubs and small trees. In the plants of this type, scalariform perforations of vessels prevail more than simple ones, considering that the former was found more scarcely in the total but almost equally found in the frequency of this type.

The type C are found in a number of herbs and trees and in a limited number of shrubs. It is notable that this type is found very frequently in lianas, especially in the herbaceous ones. It is also notable that the perforation of the vessels in such plants are simple in most cases. Lauraceae, Lardizabalaceae, Urticaceae, Moraceae, Ulmaceae, Euphorbiaceae and Geraniaceae are families which are dominated by species of this type. Leguminosae, Scrophulariaceae and Umbelliferae also possess a considerable number of species which belong to this type in the lignification.

The type D is the commonest one to which a lot of trees, shrubs and herbs belong. Trees with scalariform perforations in the vessels are scanty in this type, and most of them have been recognized as primitive (Magnoliaceae, Cercidiphyllaceae, Eupteleaceae). Among these families, Magnoliaceae possesses some vessels with simple perforations mixing with those with scalariform perforations. Betulaceae showed this type of lignification, possessing the vessels with scalariform perforation; to this family various systematic positions have been offered by students of phylogeny. Among the shrubs with scalariform perforations in the vessels, Ternstroemiaceae, Hamamelidaceae, Buxaceae and Aquifoliaceae are families which are dominated by species of this type. Among those which possess vessels with simple perforations, Menispermaceae, Salicaceae, Rosaceae, Pomaceae, Fagaceae, Malvaceae, Juglandaceae, Rhamnaceae, Acanthaceae, Verbenaceae, Labiatae, Campanulaceae, Cornaceae and Caprifoliaceae are families dominated by the species of this type. It is not rare that the species of a family are distributed in several types and the species of a family are almost equally distributed in more than one type. Thus it is not proper to classify families in a certain type absolutely.

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[1] This subclass includes Rosales, Hamamelidales, Stachyurales, Thymeleales and Myrtales.

In the xylem of angiosperms, the vessel often showed more negative colors than the other elements. The vessels which were formed earlier sometimes showed more negative colors than those which were formed more lately. The protoxylem showed negative colors in those plants tested with very few exceptions. In the cell wall, the primary wall and the earlier formed secondary wall are apt to be more negative in colors than the later formed secondary wall.

Discussion

It is doubtless that the lignin which shows the positive color with the Mäule test is phylogenically more advanced than that which shows the negative color. Although the lignin of the negative color is distributed in many advanced dicotyledons, they are located in limited kinds of tissues. Positive colors present in *Selaginella*, some species of Filicales, *Podocarpus* and *Ephedra* probably were attained independently[1]. Furthermore, the fact that few positive colored cells were found in the sections of two species of Chloranthaceae (*Sarcandra glabra* and *Chloranthus spicatus*) revealed that Mäule positive lignin may have originated after the evolution of angiosperms. Thus parallel evolution occurred in various plants as shown in the anatomical development of the vessel.

It has been accepted that there is an anatomical trend of evolution of xylem elements, from tracheids to vessels with scalariform perforations and then to vessels with simple perforations. This trend of evolution is considered to be the differentiation of xylem elements to mechanical utility on one hand, and to conductive utility on the other. When the trend of evolution in the lignification of vessel elements, the Mäule negative to the Mäule positive, is considered with reference to the anatomical trend of evolution, interesting results follow.

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[1] *Zamia*, which has been known to possess Mäule positive cells also probably attained to the Mäule positive stage independently.

In pteridophytes, the sclerenchyma in the cortex, which is of mechanical utility, is apt to be more positive in the Mäule reaction than the xylem, which is of conductive utility. Three species in *Selaginella* and one species of ferns, *Pteridium aquilinum*, which possess vessels in the xylem showed noticeable positive colors in the sclerenchym in the cortex. In gymnosperms, *Ephedra distachia*, which possess vessels, showed a positive color in the xylem.

In angiosperms, the characteristic to show the Mäule negative colors is apt to be accompanied by the characteristic to possess scalariform perforations in the vessel, and the characteristic to show the positive colors in the mechanical tissue is usually accompanied by the characteristic to possess simple perforations. Especially the type C and simple perforations are found in very high correlation. It is notable that the negative colors are often shown in the conductive tissue even in those families which have been recognized as the most advanced groups (e. g., Umbelliferae, Valerianaceae and Compositae). In monocotyledons, Gibbs reported that sometimes the Mäule reaction turned out more positive in the bundle sheath or in fibers than in the xylem.

From these facts, it follows that the anatomical evolution of conductive tissues, from tracheids to vessels with scalariform perforations and to vessels with simple perforations, is one trend of evolution in the xylem, and the development of the Mäule positive lignin in mechanical tissues is another, and that these trends are generally parallel with each other in their process of evolution. However, in the two kinds of evolution, it is usually found that one developes faster than the other even in the same plant. In Filicales which are shown to be positive, no vessel is found except in one species, *Pteridium aquilinum*. Among the three vesselless species of angiosperms observed, *Drimys aromatica*1) and *Trochodendron aralioides*2) showed positive reaction, while *Sarcandra glabra* gave negative reaction.

The fact that the frequency of the type C is very small in shrubs and that of the type B or D is great in the ratio reveals that shrubs are poor in the specialization of lignification between the mechanical tissue and the conductive

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1) In *Drimys aromatica*, a trace of negative colors was found in some samples.

2) *Trochodendron aralioides* belongs to the type B, but the negative colored part is very poor in amount. Thus, it can be admitted to account this plant as the type D in discussion.

tissue in the xylem. This shows that the development of Mäule positive lignin occurred at an approximately uniform pace among the xylem elements. This state of poor specialization coincides with the poor specialization of anatomical structure between the mechanical tissue and the conductive tissue in the xylem of shrubs and with the habit of shrubs which require neither strong mechanical strength nor good conductivity.

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摘　　　要

リグニンを含有する試料の切片を，過マンガン酸加里で酸化し，水洗後，塩酸に浸して塩素化し，再び水洗後，アンモニヤで処理後検鏡すると，シダ植物，裸子植物のもつリグニンは，黄色又は黄褐色（陰性反応）を，被子植物のものは赤色（陽性反応）を呈する。この反応はモイレ反応といわれる。著者は，植物の進化系統の追究の一助にもなろうかと，維管束植物のモイレ反応を，組織別に着目して観察した。

シダ植物に於ては，従来 *Selaginella* 属以外にはモイレ陽性反応が見出されなかったが，キジノオシダ，イワヒトデ，ヤノネシダ，アツイタ，ヒメムカゴシダおよびワラビ

に陽性反応を見出した。これらにおいて，一般に，皮層の厚膜細胞の方が，木部の細胞よりも，より陽性になる傾向がある。裸子植物では *Ephedra distachia* が顕著な陽性反応を示し，マキの材の横断面では，陽性の部分と陰性の部分とが，赤と黄の縞をなしてあらわれた。しかし，他の裸子植物は，僅かの不明瞭な例外を除きすべて陰性を呈した。372の被子植物（双子葉）を検したところ，センリョウ，チャランにおいては陽性反応を呈する細胞が殆んど認められず，更に，相当数の種に於て木部に陽性反応がみとめられなかった (Schisandraceae, Saururaceae, Chloranthaceae, Aristolochiaceae, 草木の Leguminosae 等)。導管をもたぬ被子植物は3種観察されたが，そのうちセンリョウは陰性，*Drimys aromatica*，ヤマグルマは陽性を示した。

観察された被子植物を組織別に注目し，反応によってみると，4つの主な型が木部のリグニン化に関して考えられる。即ち，A型–すべて陰性，B型–陽性と陰性の色がまざってあらわれる（一つの細胞の中にも陽性，陰性の色がまざることがある），C型–導管のみが陰性，他の部分は陽性，D型–全部陽性である。被子植物の木部に於て，導管はしばしば他の組織よりも陰性の色を示す。細胞膜のうちで一次膜及び早くできた二次膜は，新しくできた二次膜よりも陰性になりがちであった。リグニン化の型を導管の穿孔と対比して考察すると，機械組織がモイレ陰性を呈する性質は導管が階紋穿孔をもつ性質と伴なう傾向があり，機械組織がモイレ陽性を呈する性質は，導管が単穿孔をもつ性質と伴なう傾向がある。かくて，仮導管から階紋穿孔導管をへて単穿孔導管へという通導組織の解剖学的な進化と，機械組織におけるモイレ反応陰性のリグニンから陽性のリグニンへの進化は，共に多元的なものであって，概ねたがいに平行して進むが，一つの植物に於て一方の性質が他のものにくらべ先に進むこともしばしばありうるものである。

○ハイマツ北上山地片羽山に産す（小水内長太郎）Chotaro KOMIDZUNAI: *Pinus pumila* found on Mt. Kataba, northern Honshu

筆者は昭和12年頃から，北上山地片羽山雄岳（1313m）のハイマツ自生について注目していたが，たまたま林弥栄氏の“針葉樹の分類と分布”を見て，本山の報告がないことを知った。第1回は昨年7月23日（1961）に調査を行なったが，ハイマツは同山頂より約30m程下った南東斜面に約50m巾に1列にならんでいた。チシマザサ，シロバナシャクナゲ等と密に混生して，約20株あった。当時折角結ばれた毬果は，鳥類の食害をうけ，種子は一つも残されていない状況であった。8月18日の2回目の調査では，頂上下約30m東斜面に，1株望見出来たが，地形が険しく，現地調べはできなかった。本山麓青ノ木部落の八幡利男氏は，以前片羽山頂一帯はハイマツの密生地であったが，山火事のためほとんど絶滅したと語っている。これを裏書するように，山頂の所所に枯れた根株が残っている。幼生も少し見かけたが，旧に復するに長くかかるものと考える。終りにのぞみ，御教示戴いた岩手大学助教授菊地政雄氏に謝意を表します。

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